Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2014, 3 (3):1277-1281

(International Peer Reviewed Journal)

Synthesis and Antimicrobial Activity of Novel Benzohydrazide Derivatives of 2,4-Dichlorobenzoic acid

V. Laxmi Prasanna [1,2 *] and R. Narender [2]

1. Vignan Institute Of Technology & Science, Vignan hills, Deshmukhi (Village), Nalgonda (District), Hyderabad-508284**, INDIA**
2. Department of Humanities & Science, Jawaharlal Nehru Institute Of Technology, Ibrahimpatnam, Hyderabad 501 510**, INDIA**

Email: v.laxmi.prasanna.2014@gmail.com

Accepted on 23rd May 2014

ABSTRACT

*The present paper describes the synthesis and antibacterial activity of new benzohydrazide derivatives derived from 2,4-dichlorobenzoic acid and using certain heterocyclic aldehydes. The newly synthesized benzohydrazide **4a – 4g** were screened against four bacterial strains viz., Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus pyogenes. Results of the antibacterial data revealed that compounds **4d**, **4e**, **4f** and **4g** exhibited good antibacterial activity with reference to the standard drug.*

Keywords: 2,4-dichlorobenzoic acid, Antibacterial Activity, Benzohydrazide, Gram-positive bacteria, Synthesis

INTRODUCTION

The search for new antibacterial drugs is a continuous process because of the increasing resistance of microbial pathogens. It is enviable to find drugs with more potency with wide activity spectrum. The treatment of bacterial infectious diseases is a never ending challenge because of the increasing number of multi-drug microbial pathogens [1,2]. In recent years, the design of new compounds, having new structures and new targets of action, has become one of the most important areas in the antibacterial research purpose [2].

Hydrazone derivatives are molecules containing highly reactive azomethine group (CO-NH-N=CH) and thus useful in new drug development [3]. Many studies have confirmed that hydrazone derivatives show evidence of wide spectrum of biological effects including antimicrobial [4], analgesic [5], anti-platelet [6], anti-tubercular [7] and anti-tumoral [8] activities. The aim of the present paper is to report the synthesis and antibacterial activity of new benzohydrazide derivatives derived from 2,4-dichlorobenzoic acid and using certain heterocyclic aldehydes. The newly synthesized benzohydrazide compounds **4a-4g** were screened *in-vitro* at a concentration of 100 µg mL^{-1} for antibacterial activity against two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram-positive strains (*Staphylococcus*

aureus and *Staphylococcus pyogenes*) with reference to the standard antibacterial drug ciprofloxacin (100 µg / mL). The related work cited in the references[11-15]

MATERIALS AND METHODS

Chemicals and solvents used were purchased either from Fluka or Merck. All the reagents were of analytical grade. Thin-layer chromatography (TLC) was performed on E.Merck AL silica gel 60 F254 plates and visualized under UV light. The IR spectra were recorded on a Perkin Elmer FT-IR spectrometer. The ^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian EM-360 spectrometer (400MHz). The ^{13}C NMR spectra recorded in $CDCl_3$ on a Varian EM-360 spectrometer operating at 100MHz. All the chemical shifts were reported in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on Agilent ion trap MS. All the reactions were carried out under argon atmosphere.

Synthesis of ethyl 2,4-dichlorobenzoate 2 : To a solution of compound **1** (2g, 10.48 mmol) in ethanol (10 mL) was added sulphuric acid (two drops) and refluxed for 12 h. After completion of reaction, ethanol was evaporated under reduced pressure and the obtained residue was taken in ethyl acetate (25 mL) and washed with 10% aq; $NaHCO_3$ solution (2 x 15 mL) followed by water wash and brine solution. The organic layer was separated, dried over Na_2SO_4, filtered and evaporated to afford compound **2.** Yellow oily liquid; Yield: 1.95 g, 85%; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75 (d, *J* = 6.8 Hz, 1H), 7.70 (s, 1H), 7.49 (d, *J* = 6.8 Hz, 1H), 4.26 (q, *J* = 5.6 Hz, 2H), 1.24 (t, *J* = 5.6 Hz, 1H);ESI-MS: m/z, 219 (M+1).

Synthesis of 2,4-dichlorobenzohydrazide 3: To a solution of compound **2** (1.45 g**,** 6.62 mmol) in ethanol (10 mL) was added hydrazine hydrate (2.34 g, 46.54 mmol) and heated to 85°C for 3h. After completion of the reaction, ethanol was concentrated under reduced pressure to obtain crude compound **3.** The crude compound was dissolved in ethanol (20 mL) at 45°C to get clear solution and cooled to 10°C, filtered at high vacuum pump and dried to obtain compound **3.** Off-white solid; Yield: 1 g, 80%; m.p: 98-99 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.62 (s, 1H), 7.40 (d, *J* = 6.8 Hz, 1H), 7.33 (d, *J* = 6.8 Hz, 1H), 4.44 (br.s, 2H), 3.28 (br.s, 1H); ESI-MS: m/z, 206.0 (M+1).

General Experimental Procedure for the Synthesis of Hydrazones derivatives (4a-4j): To a stirred solution of compound **3** (100 mg, 0.48 mmol) in ethanol(1 mL) was added corresponding heteroaromatic aldehydes (0.53 mmol) and heated to reflux for 1h. After completion of reaction cooled to 10°C, filtered and washed with cooled ethanol and followed by pet-ether to obtain the pure compound. Yields of the products, varied between 85 to 95%.

(E)-2,4-dichloro-N'-((5-nitrothiophen-3-yl)methylene)benzohydrazide (4a): Yellow solid; Yield: 92%; m.p: 108-109 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.09 (* 11.98, s, 1H), 8.24 (* 8.04, ddd, *J* = 1.6, 1.6, 1.6 Hz, 2H), 8.21 (* 7.99, s, 1H), 7.70 (ddd, *J* = 1.6, 1.6, 1.6, Hz, 2H), 7.50 (d, *J* = 3.6 Hz, 1H), 7.48-7.41 (m, 2H); ESI-MS: m/z, 344 (M^{+1}).

(E)-2,4-dichloro-N'-((5-nitrothiophen-2-yl)methylene)benzohydrazide (4b): Yellow solid; Yield: 90%; m.p: 112-113 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.37 (* 12.23, s, 1H), 8.44 (* 8.16, s, 1H), 8.06 (* 7.69, d, *J* = 3.4 Hz, 1H), 7.66 (d, *J* = 6.8 Hz, 1H), 7.58 (* 7.48, d, *J* = 6.4 Hz, 1H), 7.53-7.39 (ser.m, 2H); ESI-MS: m/z, 344 (M+1).

(E)-2,4-dichloro-N'-((5-methylfuran-2-yl)methylene)benzohydrazide (4c): Yellow solid; Yield: 84%; m.p: 140-141 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.78 (* 11.72, s, 1H), 7.96 (* 7.79, s, 1H), 7.70 (* 7.64, s, 1H), 7.54-7.38 (m, 2H), 6.77 (* 6.59, s, 1H), 6.20 (* 6.11, s, 1H); ESI-MS: m/z, 297 (M+1).

(E)-N'-((benzofuran-2-yl)methylene)-2,4-dichlorobenzohydrazide (4d): Yellow solid; Yield: 88%; M.p: 122-123 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.18 (br.m, 1H), 8.32 (* 8.13, s, 1H), 7.80 (* 7.65, s,

1H), 7.77-7.72 (m, 1H), 7.68-7.66 (m, 1H), 7.58-7.52 (m, 2H), 7.46-7.24 (ser.m, 3H); ESI-MS: m/z, 333 (M^{+1}).

(E)-2,4-dichloro-N'-((pyridin-4-yl)methylene)benzohydrazide (4e): Pale yellow solid; Yield: 90%; m.p: 118-119 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.24 (* 12.13, s, 1H), 8.70 (d, *J* = 4.4 Hz, 1H), 8.48 (d, *J* = 4.4 Hz, 1H), 8.20 (* 8.0, s, 1H), 7.68 (dd, *J* = 4.2, 7.2 Hz, 1H), 7.60-7.56 (m, 1H), 7.52-7.44 (m, 1H), 7.24 (d, *J* = 4.6 Hz, 1H); ESI-MS: m/z, 294 (M^{+1}).

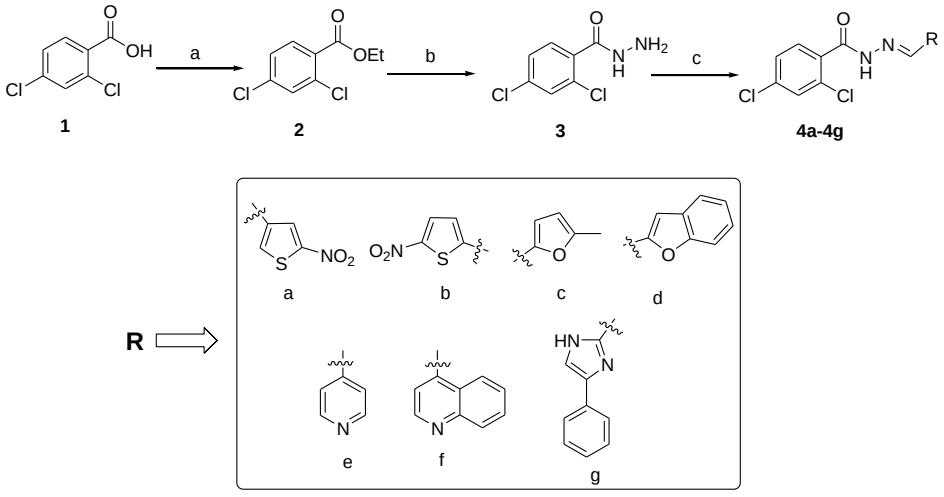
(E)-2,4-dichloro-N'-((quinolin-4-yl)methylene)benzohydrazide (4f): Yellow solid; Yield: 80%; m.p: 120-121 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.44 (* 12.34, s, 1H), 8.92 (ddd, *J* = 2.8, 2.8, 6.8 Hz, 1H), 8.90 (* 8.56, s, 1H), 8.12 (ddd, *J* = 6.4, 6.4, 6.8 Hz, 1H), 7.84 (d, *J* = 6.4 Hz, 2H), 7.78 (br.t, *J* = 6.2 Hz, 1H), 7.72 (d, *J* = 6.8 Hz, 1H), 7.62 (s, 1H), 7.54 (d, *J* = 2.8 Hz, 1H), 7.40 (br.t, *J* = 6.0 Hz, 1H); ESI-MS: m/z, 344.1(M^{+1}).

(E)-2,4-dichloro-N'-((4-phenyl-1H-imidazol-2-yl)methylene)benzohydrazide (4g): Yellow solid; Yield: 80%; m.p: 120-121 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.15 (* 12.03, s, 1H), 8.37 (* 8.11, s, 1H), 8.30 (d, *J* = 11.2 Hz, 1H), 7.89-7.52 (ser.m, 8H), 7.20 (d, *J* = 14.0 Hz, 1H); ESI-MS: m/z, 359.2 (M^{+1}).

RESULTS AND DISCUSSION

Chemistry: The 2,4-dichlorobenzoic acid was converted to its corresponding ethylbenzoate derivative **2**, using catalytic qty; of conc;H2SO4 in ethanol at reflux for 12 h. The ethyl ester derivative was further treated with hydrazine hydrate in ethanol at 85 ^{o}C for 3h resulted in formation of the key intermediate **3**. The condensation of compound **3** with various heterocyclic aldehydes gave various new hydrazone derivatives **4a- 4g** in quantitative yields. The reaction progress of these derivatives is depicted in Scheme **1.**

The structures of the synthesized compounds were confirmed by ^{1}H NMR and Mass spectral data. All the synthesized hydrazones derivatives **4a-4g** compounds were found to exist as a mixture of two rotameric forms in solution [9] e.g. antiperiplanar (*ap*) and synperiplanar (*sp*) as indicated by their ^{1} H NMR spectra. Two sets of signals were observed for all groups in the ^{1}H NMR spectra of each compound indicating the possibility of equilibrium and interconversion between rotamers (and/or configurational isomers) in solution [9].



Experimental Conditions: a) Conc; H_2SO_4, ethanol, reflux, 12 h; b) $NH_2NH_2.H_2O$, ethanol, 85 ^{o}C, 3 h; c) Hetero aromatic aldehydes **a-g**, ethanol, reflux, 1 h

Scheme 1. Synthesis of Hydrazone derivatives **4a – 4g**

Antimicrobial Activity : All the newly synthesized hydrazone compounds **4a-4g** were screened *in-vitro* at a concentration of 100 µg/mL for antibacterial activity against two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram-positive strains (*Staphylococcus aureus* and *Staphylococcus pyogenes*). The antimicrobial activity was determined using disc diffusion method by measuring zone of inhibition in mm [10]. Standard antibacterial drug ciprofloxacin (100 µg / mL) was also tested under similar conditions against these organisms. Each experiment was done in triplicate and the average reading was taken. The results of antibacterial activity evaluation are presented in **Table 1**.

Table 1: Antibacterial Activity of Benzohydrazide derivatives 4a-4g

Compound No	Gram negative bacteria		Gram positive bacteria	
	Ec	Pa	Sa	Sp
	Zone of inhibition			
4a	22	19	17	16
4b	23	20	16	16
4c	22	18	17	17
4d	25	24	19	20
4e	25	25	20	20
4f	26	24	20	21
4g	26	25	21	21
* Ciprofloxacin	28	26	21	22

Ec: Escherechia coli; Pa: Pseudomonas aeruginosa; Sa: Staphylococcus aeruginosa; Sp: Staphylococcus pyogenes; * : Conc. 100 µg mL^{-1}

From the **Table 1,** it is observed that among all the compounds tested, compound 4**a, 4b** and **4c** showed moderate antibacterial activity (zone of inhibition: 18-23 mm), while the compounds **4d, 4e, 4f** and **4g** exhibited good activity (zone of inhibition: 25-26 mm) against Gram positive bacterial strains. In case of Gram negative bacteria, compounds **4d – 4g** exhibited good antibacterial activity while compounds **4a - 4c** showed moderate antibacterial activity.

APPLICATIONS

Some of the newly prepared benzohydrazide derivatives **4a-4g** in the present paper showed good antibacterial activity. These derivatives can be tested further on the various available bacterial strains.

CONCLUSIONS

In conclusion, the newly synthesized benzohydrazide derivatives **4a – 4g** described in this paper was prepared from commercially available 2,4-dichlorbenzoic acid and screened against two Gram positive and two Gram negative bacterial strains. The structures of the synthesized compounds were confirmed by ^{1}H NMR and Mass spectral data. Compounds, **4d, 4e, 4f and 4g** displayed good antibacterial activity while compounds **4a, 4b and 4c** showed moderate antibacterial activity.

REFERENCES

[1] Diana Camelia Nuță, Carmen Balotescu Chifiriuc, Alexandru Vasile Missir, Ileana Cornelia Chiriță, Carmellina Daniela Bădiceanu. *Farmacia.*, **2010**, 58, 38-45.

[2] Ioana Moş, Otilia Micle, Mihaela Zdrâncă, Mariana Mureşan, Laura Vicaş. *Farmacia.*, **2010**, 58, 637-645.

[3] O. A. Olayinka, A. O. Craig, C. N. Obinna, A. A. David. *Bioorg.Med.Chem.*, **2010,** 18, 214-221.

[4] A. Cukurovali, B. Yilmaz, S. Gur and C. Kazaz. *Eur.J.Med.Chem.*, **2006**, 41, **2006**, 201-207.

[5] P. C. Lima, L. M. Lima, K. C. Silva, P. H. Leda, A. L. P. Miranda, C. A. M. Fraga, E. J. Barreiro, *Eur.J.Med.Chem.*, 2000, 35, **2000**,187-203.

[6] G. A. Silva, L. M. M. Costa, F. C. B. Brito, A. L. P Miranda, E. J. Barreiro, C. A. M. Fraga, Bioorg.Med.Chem. **2004**, 12, 3149.

[7] L. C. du Toit, V. Pillay, M. P. Danckwerts. Respiratory Research, **2007**, 118.

[8] A. M. El-Hawash, W. A. E Abdel and M. A. El-Dewe- llawy. Archiv der Pharmazie., **2006**, 339, 14-23.

[9] N. Rajasekhar, K.B. Chandrasekhar, M. Sandeep, B. Balram. *Letters in Drug Design & Discovery.*, **2013**, 10, 620-624.

[10] A. N. Bauer, W. N. M. Kirby, J. C. Sherries, M. Truck, *Am. J. Clin. Pathol.*, **1996**, 45, 493.

[11] Battula Sreenivasa Rao, Gurrala Srinivasa Rao, B.V.Kiran, K.Siva Nagaraju. Effect of Nonionic Surfactant (Triton X–114) on the Spectrophotometric Determination of Selenium (IV) With Isonitriso p-Isopropyl Acetophenone Phenyl Hydrazone. *Journal of Applicable Chemistry,* **2012**, 1 (2), 232-238.

[12] K. Siddappa, Mallikarjun Kote. Synthesis, Characterization and Antimicrobial studies of N-1-(1-(2-Hydroxy-5-Methylphenyl) Ethylidene)-2-Oxo-2H-Chromene-3-Carbohydrazide and its Metal Complexes. *Journal of Applicable Chemistry*, **2013**, 2 (3), 405-414.

[13] T. Ravi Sankar, P. Venkata Ramana. Synthesis, Characterization and Applications of Polymer-metal chelates derived from Poly(4-acryloxybenzaldehyde)-divinylbenzene salicyloyl hydrazone resins. *Journal of Applicable Chemistry*, **2013**, 2 (6), 1459-1467.

[14] Rajasekhar Narisetty, K.B. Chandrasekhar, Sandeep Mohanty, M. Rameswara Rao, B. Balram. Synthesis and Antimicrobial Evaluation of Some Novel Hydrazone Derivatives of 2,5-iflurobenzoic acid. *Journal of Applicable Chemistry*, **2013**, 2 (6), 1489-1498.

[15] Shaik Khadar Yazdan*, K. Sampath Kumar, B. Ratnabhushanam, J. V. Padmavathi, G. Krishna Kishore, K. Mohan. Synthesis of Acetophenone Chalcone Derivatives And their Antibacterial Activity. *Journal of Applicable Chemistry*, **2014**, 3 (2), 601-605.